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DOI: https://doi.org/10.1016/j.ijfoodmicro.2013.05.012

Running head: Detection of *Salmonella* by Real-time PCR

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Abstract

The traditional cultural detection of *Salmonella* spp. is both time- and labour-intensive. *Salmonella* is often a release criterion for the food industry and time to result is therefore an important factor. Storage of finished products and raw materials can be costly and may adversely impact available shelf-life. The application of real-time PCR for the detection of *Salmonella* spp. in food samples enables a potential time-saving of up to four days. The advancement of real-time PCR coupled with the development of commercially available systems in different formats has made this technology accessible for laboratories in an industrial environment. Ideally these systems are reliable and rapid as well as easy to use.

The current study represents a comparative evaluation of seven commercial real-time PCR systems for the detection of *Salmonella*. Forty-nine target and thirty-two non-target strains were included in the study to assess inclusivity and exclusivity. The limit of detection for each of the method was determined in four different food products. All systems evaluated were able to correctly identify the 49 *Salmonella* strains. Nevertheless, false positive results (*Citrobacter* spp.) were obtained with four of the seven systems. In milk powder and bouillon powder, the limit of detection was similar for all systems, suggesting a small matrix effect with these samples. Conversely, for black tea and cocoa powder some systems were prone to inhibition from matrix components.

Keywords: commercial real-time PCR systems, *Salmonella* detection, specificity, detection limit

1. Introduction

The ISO standard cultural method for the detection of *Salmonella* spp. involves a non-selective pre-enrichment in buffered peptone water, followed by a selective enrichment in Rappaport-Vasilliadis Soya broth and Muller-Kauffmann Tetrathionate-Novobiocin broth and plating onto selective and differential agars. Two selective solid media are inoculated; xylose lysine deoxycholate agar (XLD agar) and any other solid selective/differential medium of choice that is complementary to XLD. Presumptive positive colonies must then be confirmed biochemically and serologically (ISO 6579:2002). It can take up to 5 working days to receive a confirmed positive result. Although still considered as the "gold standard", cultural methods are labour-intensive and time-consuming. The application of molecular based methods, such as real-time PCR, can significantly reduce the manpower and time required to detect pathogens such as *Salmonella*. In recent years, increasingly rapid and specific PCR based methods have been developed to identify *Salmonella* contamination in environmental and food samples and to confirm the identity of isolated *Salmonella* cultures.

Even though PCR is a valuable tool for scientists, allowing for fast and effective analysis of samples from different origins, inhibition is the most common reason of PCR failure when adequate copies of DNA are present (Alaeddini, 2011). Inhibition of PCR can be caused by various compounds present in the food matrix, in the growth media and in the reagents used for extraction. Rossen and co-workers (1992) tested a wide range of components for the maximum amount that can be added to a PCR reaction without causing inhibition. Their findings showed that concentrated protein, unrelated DNA and levels of $MgCl_2$ potentially have a negative effect on PCR sensitivity. PCR inhibition mechanisms can be grouped into three categories: inhibiting substances can affect cell lysis during DNA extraction, capture or degrade nucleic acids or interfere with the *Taq* DNA polymerase activity (Kontanis et al., 2006). Known PCR inhibitors include proteinases, calcium ions, polyphenolics, tannins,

humic acid, complex polysaccharides, collagen, bile salts, heme, haemoglobin, myoglobin, urea, lactoferrin, immunoglobulin G, melanin and eumelanin (Rådström et al., 2004). Approaches for the prevention of inhibition will include either a clean-up of the extracted DNA or a dilution of the sample. However, dilution of the sample requires laborious sample manipulation and may result in template depletion if template DNA concentrations are low (Kontanis et al., 2006). DNA clean- up kits often include a filter column that removes inhibitors like polyphenolics, humic acids, tannins etc. from nucleic acid solutions with little work effort and minimal impact on DNA concentration.

The establishment of real-time PCR and commercialization of detection kits facilitates application of PCR for routine testing. However, evaluation data of these systems are so far very limited and restricted to the testing of single systems (Bennet et al., 1998, Cheung et al., 2004). Therefore, the objective of the present study was to evaluate in parallel seven commercially available real-time PCR based systems for the detection of *Salmonella*. A limit of detection (LOD) approach was used with food samples known to be challenging for PCR, e.g. containing inhibitory compounds. In addition, for food matrices where inhibition of the PCR reaction was apparent, the performance of different extraction methods for sample clean up prior to PCR were compared. Moreover, the tests were also compared in view of their convenience and applicability for use in a routine testing laboratory.

2. Materials and methods

2.1. Food samples

Skimmed milk powder, a bouillon powder containing Mediterranean herbs, black tea and cocoa powder were used in the study. These food samples were chosen, as they are known to contain compounds that can potentially inhibit the PCR reaction.

2.2. Bacterial strains

A total of 49 *Salmonella enterica* subsp. *enterica s*trains, covering a range of 39 serotypes, and 29 non-*Salmonella* were used for inclusivity and exclusivity testing respectively. The non-*Salmonella* strains included species with similar growth requirements and typically isolated from similar sources to *Salmonella*. The strains were grown on plate count agar (PCA, Oxoid) at 37 °C for 24 hrs. The bacterial strains were stored at 4 °C in ½ tryptose soy agar (TSA, Oxoid).

Salmonella Tennessee strain S511, an isolate from a pet food factory in France, was selected for the LOD experiments. Previous experiments confirmed that this strain can be detected with all PCR systems evaluated. One colony of S511 grown on PCA was transferred to 5 mL of brain heart infusion broth (BHI, Oxoid) and incubated overnight at 37 °C. The cell counts of the overnight culture were determined by plate counting on PCA. Before spiking of the sample, the overnight culture was diluted in physiological saline solution (0.9 % NaCl) to achieve the desired inoculation level.

2.3. Inoculation and enrichment

A total of 22 portions (25 g) of each food type were weighed aseptically into stomacher bags and the appropriate enrichment diluent was added. The 25 g samples of skimmed milk

121 powder were enriched in 225 mL of buffered peptone water (BPW, Oxoid). Cocoa powder
122 was enriched in 225 mL of skimmed milk supplemented with brilliant green to a final
123 concentration of 0.018 g/L. The 25 g portions of black tea and bouillon powder with
124 Mediterranean herbs were each enriched in 900 mL of BPW; this higher dilution is routinely
125 used in commercial testing laboratories as it has been found to be necessary to prevent growth
126 inhibition of the target organism in these matrices. For the LOD experiments the sample
127 homogenates were inoculated with 100 µL of the diluted inoculum in different concentrations.
128 Six replicate samples were spiked with one of three inoculums to give estimated counts of 0.3
129 CFU/25 g, 1 CFU/25 g, or 3 CFU/25 g. Two replicates, inoculated with ca. 10 CFU/25 g
130 served as a positive control. Two replicates were not inoculated to serve as negative controls
131 and to detect if the sample matrices caused false positive results, e.g. due to auto-
132 fluorescence. The samples were mixed by kneading the stomacher bag for approximately 20
133 seconds by hand and incubated at 37 °C for 16 to 18 hours. The concentration of the inoculum
134 was estimated by plating on PCA.
135
136 2.4. Detection systems evaluated
137 The current study represents a comparative evaluation of seven diagnostic systems namely
138 ADIAFOOD® *Salmonella* (AES chemunex, Bruz cedex, France) applied on the Stratagene
139 MX3005P, BAX® system Q7 real-time *Salmonella* (DuPont Qualicon, Wilmington, USA)
140 applied on the BAX system Q7, BIOTECON foodproof© *Salmonella* Detection Kit (Biotecon
141 Diagnostics, Potsdam, Germany) applied on the Lightcycler® 2.0, BioControl Assurance
142 GDS® TM *Salmonella* (BioControl, Bellevue, USA) applied on the GDS Rotor Gene®,
143 Genedisc® Shiga Toxic *E. coli* and *Salmonella* spp. (Pall GeneDisc® Technologies, Bruz,
144 France) applied on the GeneDisc® Cycler, BioRad iQ-Check® *Salmonella* 2 (Biorad,
145 Marnes-la-Coquette, France) applied on the MiniOpticon™ and MicroSeq® *Salmonella* spp.

146 Detection kit (Applied Biosystems, Foster City, USA) applied on the 7500 Fast Real-Time
147 PCR System.
148
149 2.5. DNA extraction
150 2.5.1. Inclusivity/exclusivity
151 Strains used for inclusivity/exclusivity testing were grown overnight on PCA at 37 °C. One
152 colony from PCA was added to 450 µL of lysis buffer comprising 20 mL TrisHCL pH 8.5 1
153 M (12.1 g Tris-(hydroxymethyl)- Aminomethan (Sigma- Aldrich, Buchs, Switzerland),
154 distilled water to 100mL); 100 µL Tween 20 (Merck, Darmstadt, Germany); 48 mg Proteinase
155 K (Sigma- Aldrich, Buchs Switzerland, P6556), distilled water to 200 mL and heated for 40
156 minutes at 60 ºC ± 1 ºC followed by 20 minutes at 95 ºC± 1 ºC in a heating block
157 (Thermomixer Comfort, Eppendorf). Subsequently the lysate was added to the PCR reaction
158 according to the volume of sample recommended to be added to the assay reagents by the
159 individual manufacturer's instructions. For the BAX® system Q7 real-time *Salmonella* assay,
160 a colony from PCA was added to 5 mL brain heart infusion broth (BHI) and incubated
161 overnight at 37 °C. The extraction was then performed as described in the manufacturer`s
162 protocol.
163
164 2.5.2 Comparison of commercial DNA extraction/DNA clean-up kits
165 In addition to the use of the proprietary methods proposed by the RT-PCR kit manufacturers,
166 different commercially available sample preparation and nucleic acid clean up kits were
167 evaluated for their ability to overcome the PCR inhibition caused by certain food matrices.
168 Manual extraction and clean-up kits from both PCR and non PCR manufacturers were
169 evaluated, including PrepSEQ™ Rapid Spin Sample Preparation Kit and PrepSEQ™ Rapid
170 Spin Sample Preparation Kit – Extra Clean (Applied Biosystems), foodproof® StarPrep one
171 Kit (Biotecon), the Food Extraction Pack 01 (Pall GeneDisc® Technologies), DNEasy®

172 *mericon* Food Kit, both 2g and 200g protocols, (Qiagen, Hombrechtikon, Switzerland),
173 Masterpure™ Complete DNA and RNA purification kit (Epicentre, Madison, USA) and
174 OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA). In addition two
175 automated systems, MagMAX™ Express-96 Magnetic Particle Processor (Applied
176 Biosystems) and QIAsymphony SP (Qiagen) were also included in the comparison.
177 Matrices known to contain inhibitory compounds (tea, chocolate and Mediterranean herb
178 mix) were inoculated with high levels of *Salmonella* (10^4 CFU/25 g) and enriched overnight
179 in BPW as described above along with an uninoculated control sample. The CFU/mL of the
180 inoculated sample after enrichment was estimated using plate counts on XLD agar to ensure
181 the *Salmonella* had grown. Duplicate 1 mL aliquots from the uninoculated control sample and
182 duplicate aliquots from the inoculated samples were subjected to heat lysis at 97 ± 2 ºC with
183 no inhibitor removal step. Aliquots from the inoculated sample were also processed in
184 duplicate according to the instructions for each of the commercial extraction methods. The
185 DNA and protein concentrations of each of the extracts were measured using a
186 spectrophotometer (BioPhotometer, Eppendorf). Aliquots from each of the extractions were
187 diluted to equivalent DNA concentrations. Each of the diluted and undiluted extracts were
188 tested using the BIOTECON foodproof© *Salmonella* Kit, BioControl GDS® *Salmonella* kit,
189 Genedisc® Shiga Toxic *E. coli* and *Salmonella* spp. kit and MicroSeq® *Salmonella* kit on
190 their respective PCR cyclers. The CT values for each extraction method were compared. In
191 addition, the ease of use of each extraction method was evaluated, including cost, time-to-
192 result and hands-on time.
193
194 A follow-up evaluation of Pall GeneDisc® Food Extraction Pack 01, Applied Biosystems
195 PrepSEQ™ Rapid Spin Sample Preparation Kit and Zymo OneStep™ PCR Inhibitor
196 Removal Kit involved extraction of DNA from a range of samples including different
197 varieties of teas, coffees, chocolates, nuts, vegetables, fresh produce, herbs and spices

198 (n=107). The extraction methods were applied alone and in combination. Inhibitor removal
199 was evaluated using BioControl GDS®, PALL Genedisc® Technologies and Applied
200 Biosystems MicroSeq® kits on their respective PCR cyclers.
201
202 2.5.2. Proprietary DNA extraction from inoculated food samples
203 DNA from enriched samples was extracted using the proprietary extraction method of the
204 PCR kit or with the method recommended by the supplier. A separate extraction kit is not
205 supplied with BAX® system Q7 real-time *Salmonella,* Assurance GDS® TM *Salmonella* and
206 iQ-Check® *Salmonella* 2, however an extraction step is an integral part of these methods. For
207 the other systems the following extraction kits were recommended and used according to
208 manufacturer`s instructions: PrepSEQ™ Rapid Spin Sample Preparation Kit (Applied
209 Biosystems), foodproof® Sample Preparation Kit I (Biotecon Diagnostics) and the Extraction
210 Pack Food 01 (Pall GeneDisc® Technologies). In some cases the suppliers gave special
211 recommendations deviating from the instruction manual. For DNA extraction from milk
212 powder with the ADIAFOOD® *Salmonella* the first centrifugation steps were excluded,
213 whereas for the extraction from cocoa powder and black tea an additional washing step was
214 advised. An additional washing step was also performed for the cocoa powder and tea
215 samples with the MicroSeq® *Salmonella* spp. detection kit.
216
217 2.6. Real-time PCR amplification
218 The PCR amplifications were carried out as described in the manufacturer`s instructions. For
219 inclusivity/exclusivity testing each strain was tested once unless there was a false positive,
220 false negative or invalid result. In this case the extract of the strain was tested again in
221 duplicate. For the testing of the artificially inoculated food samples, the performance of each
222 PCR system was first evaluated using the extract of the recommended proprietary extraction
223 method. For cocoa powder and black tea, extracts that exhibited inhibition were retested after

dilution. In addition, based on results from the comparison of extraction methods, a combined extraction protocol of Extraction Pack Food 01 followed by Zymo OneStep™ PCR Inhibitor Removal kit was also used and tested on each PCR system. Initial experiments showed that the BAX® system Q7 real-time *Salmonella* and the Assurance GDS® TM *Salmonella* method did not function with extracts other than from their proprietary extraction procedures.

2.7. Data analysis

The amplification results were analysed using the software provided by the kit supplier. All PCR systems included an internal positive control that would indicate possible inhibition problems during the reaction. For the inclusivity/exclusivity testing the positive/ negative calls of the PCR software were used as a final result. Percentage inclusivity and exclusivity was calculated for the *Salmonella* and non-*Salmonella* strains respectively. Based on the PCR results of the inoculated matrices the limits of detection were calculated for each PCR system with each matrix and extract as per Wilrich and Wilrich (2009).

3. Results and Discussion

3.1. Inclusivity/exclusivity

All PCR systems evaluated were able to correctly identify the 49 *Salmonella* strains (table 1). Serotypes tested were isolated from food products or from clinical specimens and were previously used for evaluating cultural methods for *Salmonella* detection. A total of 29 non-*Salmonella* isolates were analysed with each PCR system. BAX® system Q7 real-time *Salmonella*, foodproof© *Salmonella* Detection Kit and MicroSeq® *Salmonella* spp. detection kit gave 100% negative results for these strains. Four systems, namely the ADIAFOOD® *Salmonella*, Genedisc® Shiga Toxic *E. coli* and *Salmonella* spp., Assurance GDS® TM

Salmonella and iQ-Check® *Salmonella* 2 incorrectly identified *Citrobacter murliniae* strains as *Salmonella* (table 2).

The *Citrobacter* spp. strains, which gave false positive results with certain systems, were isolated from fresh vegetables and were identified using both MALDI-TOF MS and 16S rDNA sequencing as *Citrobacter murliniae.* The problem of false positive results caused by *Citrobacter* strains in commercially available real-time PCR systems for the detection of *Salmonella* spp. has not been reported previously. However, the close relationship of the two genera is known to be a challenge for cultural and biochemical differentiation (Bennett et al., 1999). The kit manufacturers of the relevant systems are currently working towards improvement of their tests with regards to *Citrobacter* exclusivity.

3.2. *Salmonella* detection in different food matrices

3.2.1 Comparison of commercial DNA extraction/DNA clean-up kits

Salmonella counts from the inoculated samples were in the range 4.5 x 10^8-1.5 x 10^9 CFU/mL, indicating that there was no growth inhibition of the *Salmonella* during the enrichment. The automated DNA extraction systems are advantageous for processing large numbers of samples in parallel. Of the manual extraction kits, the purest DNA extract was obtained with the Masterpure™ Complete DNA and RNA purification kit (Epicentre). However, this was the most expensive, the most labour- intensive and had the longest time to result. The least labour- intensive of the manual extraction kits, with also the fastest time to result and lowest cost, was the OneStep™ PCR Inhibitor Removal Kit (Zymo Research). RT-PCR detection of *Salmonella* in the heat- lysed extracts from the uninoculated and inoculated samples was unsuccessful with indication of IPC inhibition or questionable amplification. *Salmonella* was detected in the inoculated sample using the different extraction methods with CT values in the range of 7-30 depending on the extraction method and on the RT-PCR

detection method used. The difference in CT values was related to the DNA concentration in the extracts and the volume of template used in the particular RT-PCR assays. In these experiments there was no evidence of inhibitor compounds affecting the PCR amplifications indicating that all extraction methods successfully removed sufficient amounts of inhibitors. The pattern of CT values for each of the extracts was similar across all RT-PCR systems indicating that there was no relationship between performance of the extraction method and performance of the RT-PCR method (data not shown).

Pall GeneDisc® Food Extraction Pack 01, Applied Biosystems PrepSEQ™ Rapid Spin Sample Preparation Kit and Zymo OneStep™ PCR Inhibitor Removal Kit were chosen for follow-up evaluation based on cost, ease of use and time-to-result. Successful PCR results were obtained with the majority of the 107 samples following the application of GeneDisc® Food Extraction Pack 01. For several varieties of tea the most successful results were obtained using a combination of GeneDisc® Food Extraction Pack 01 followed by additional application of Zymo OneStep™ PCR Inhibitor Removal Kit to the extract.

3.2.2 PCR inhibition with proprietary methods

With the black tea matrix, inhibition problems were apparent for the GeneDisc® Shiga Toxic *E. coli* and *Salmonella* spp. (40.9 % inhibition), iQ-Check® *Salmonella* 2 (100 % inhibition) and MicroSeq® *Salmonella* spp. detection kit (63.6 % inhibition) systems despite an extensive DNA extraction with the PrepSEQ™ Rapid Spin Sample Preparation Kit. The application of a 1:10 dilution to extracts from the MicroSeq® *Salmonella* spp. and iQ-Check® *Salmonella* 2 kits decreased inhibition to 0 % and 13.6 % respectively. However, dilution of samples is not ideal because the target DNA may be diluted to a level below the limit of detection (Kontanis et al., 2006). A clean-up of extracts from the Extraction Pack Food 01 with the Zymo OneStep™ tubes was found to reduce inhibition from the tea samples

302 ran on the GeneDisc®, iQ-Check® and MicroSeq® systems to 9.1 %, 54.5 % and 18. 2 %
303 respectively.
304
305 Almost all cocoa powder samples were inhibited when the proprietary extraction methods of
306 ADIAFOOD® *Salmonella* and iQ-Check® *Salmonella* 2 were used (90.9 % and 100 %
307 respectively). A dark colour was observed in the DNA samples, potentially indicating the
308 presence of inhibitors. Less inhibition was observed with Extraction Pack Food 01 extracts
309 from cocoa samples following a clean-up with the Zymo OneStep™ tubes (0 % inhibition for
310 ADIAFOOD® *Salmonella* and 4.5 % for iQ-Check® *Salmonella* 2).
311
312 3.2.3 Limits of detection (LOD_{50})
313 The LOD_{50} (CFU/g) for *Salmonella* Tennessee strain S511 in the four different food matrices
314 as determined with the extracts of the proprietary extraction methods are summarized in table
315 3. For black tea and cocoa powder, the LOD_{50} (CFU/g) are also given for the extracts using
316 the Extraction Pack Food 01 combined with Zymo OneStep™. For the milk powder and the
317 bouillon powder samples, values for the limit of detection were similar for all methods.
318 *Salmonella* was detected at low levels in these matrices without inhibition problems despite
319 the presence of lipids/proteins and tannins/phenolics, respectively. The use of combined
320 extraction with Extraction Pack Food 01 followed by Zymo OneStep™ tubes, compared to
321 use of proprietary extraction with/without dilution, did not provide consistent improvements
322 in LOD_{50} (CFU/g) for black tea and chocolate samples. However the amount of sample used
323 for proprietary extraction and the amount of DNA extract added to the PCR reaction differed
324 between methods and the amount of template added from the combined extraction could not
325 be normalized for direct comparison. PCR assays need to be developed in conjunction with
326 specific extraction protocols for these difficult matrices.
327

328 According to microbiological criteria for foodstuffs, methods for the isolation of *Salmonella*
329 aim at detecting 1 CFU of *Salmonella* in 25 g of product. This equates to an LOD_{50} of 0.04
330 CFU/g. In milk powder, the ADIAFOOD® *Salmonella*, Genedisc® Shiga Toxic *E. coli* and
331 *Salmonella* spp., MicroSeq® *Salmonella* spp. Detection kit and iQ-Check® *Salmonella* 2
332 gave an LOD_{50} of less than 0.04. With bouillon powder, only the MicroSeq® *Salmonella* spp.
333 Detection kit gave an LOD_{50} of less than 0.04. Following a 10-fold dilution of black tea
334 extracts, the ADIAFOOD® *Salmonella*, MicroSeq® *Salmonella* spp. Detection kit and iQ-
335 Check® *Salmonella* 2 were able to detect less than 0.04 CFU/g. The BAX® system Q7 real-
336 time *Salmonella* and foodproof© *Salmonella* Detection Kit methods were also capable of
337 detecting 0.04 CFU/g or less. BAX® system Q7 real-time *Salmonella*, Genedisc® Shiga
338 Toxic *E. coli* and *Salmonella* spp.and iQ-Check® *Salmonella* 2 gave detection limits below
339 0.04 CFU/g with cocoa powder samples. Following a 10-fold dilution of cocoa extracts,
340 ADIAFOOD® *Salmonella* detected less than 0.04 CFU/g.
341
342 Regarding the food matrices tested in this work, inhibition was most probably caused by
343 polyphenols as both black tea and cocoa powder contain high levels of these substances.
344 Tannins, which are oligomeric compounds with free phenolic groups, can oxidize to form
345 quinones that bind to the *Taq* DNA Polymerase and inactivate the enzyme, resulting in
346 decreased amplification efficiency (Young et al., 1992). The fact that inhibition was
347 significantly minimized by the application of the Zymo OneStep™ clean- up tubes implies
348 that the filter successfully retained inhibitory substances. DNA purification is recommended
349 when testing samples that contain a high content of inhibitory substances such as food
350 constituents and environmental compounds (Bessetti, 2007, Wilson et al., 1997). In this study,
351 it was shown that a DNA clean-up step can significantly reduce inhibition in samples from tea
352 and cocoa powder.
353

354

355 3.3. Convenience of use

356 With real-time PCR systems continuously replacing cultural *Salmonella* testing in
357 commercial laboratories, it is essential that the methods are efficient, fast and easy to use.
358 Ideally a minimum number of handling steps, for example opening of tubes, pipetting steps
359 and centrifugation, should be required to perform a test. Moreover, there should be little
360 danger of cross contamination when several samples are processed at a time.

361

362 The number of required centrifugation and pipetting steps varied across the platforms tested
363 in this study. Methods requiring several centrifugation steps or manual IMS
364 (immunomagnetic separation) with a lot of extraction consumables and reaction tubes were
365 regarded as laborious and not particularly user friendly. These protocols placed a limitation
366 on the number of samples that could be processed at one time and were considered quite
367 complex for high throughput routine analysis.

368

369 Advantages associated with some of the systems investigated in this study included the ability
370 to perform DNA extraction in the PCR cycler without the requirement for additional heating
371 equipment. Some systems provide reaction tubes pre-filled with dehydrated reagents, the
372 addition of the template being the only requirement. This type of system offers both
373 timesaving and the potential to prevent false results due to pipetting errors or contamination
374 of reagents.

375

376 Differences were also noticed in the flexibility of the systems. If a method can be run on more
377 than one type of real-time PCR instrument, this may be seen as advantageous. For most of the
378 kits tested here, the proprietary instrument must be purchased. Some platforms are completely

379 closed systems where analysis of the acquired data or change of the temperature profile is not
380 possible for the user.
381
382 In conclusion, this study shows that there are a number of newly developed commercially
383 available real-time PCR platforms for the detection of *Salmonella* spp., which allow rapid
384 detection of low levels of *Salmonella* in complex matrices. *Citrobacter* spp. were shown to be
385 of continued concern even with molecular detection methods for *Salmonella*. Increased
386 availability of diverse bacterial genomes will aid improvements to the design of molecular
387 probes, however it is a constant challenge to include all potential competitive organisms
388 during method development and cooperation with method users expands access to natural
389 food isolates. With the increasing availability and affordability of improved nucleic acid
390 extraction procedures and advent of automated technologies which are capable of providing
391 results with minimal manual intervention, PCR based technologies are poised to find even
392 more usage in the food industry.
393

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